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ORIGINAL ARTICLE

Vitamin B12 and folate levels increase during treatment of iron deficiency anaemia in young adult woman

Running Head

Vitamin B12 and folate and iron deficiency anaemia.

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Abstract

Introduction: The relationship between iron deficiency and vitamin B12 and folate was recognized several decades ago. Combined deficiency is important in clinical practice owing to its relationship with malabsorption syndromes. By contrast, iron deficiency and low levels of serum vitamin B12 with normal metabolic markers were often found mostly in young adults. In this work vitamin B12/folate changes were investigated during treatment of iron deficiency anemia (IDA) with pharmacological iron in young adult women.

Methods: A cohort of 35 young adult women with IDA were treated with oral iron An haematological response was obtained in 97.2 % at 4-month follow-up. Changes in serum vitamin B12, serum folate, and other biochemical parameters were monitored.

Results: Treatment with iron increased significantly serum folate and vitamin B12 from baseline. This increase was also observed in vitamin B12 levels ≤200 pmol/l (6 patients, 17.1%), in whom serum vitamin B12 was above 200 pmol/l at the end of the study in all cases. Other biochemical parameters also changed. Significant increases were seen for glucose ($p=0.012$), uric acid ($p<0.001$), total cholesterol ($p=0.023$), HDL-cholesterol ($p=0.026$), and bilirubin ($p<0.001$). Urea decreased significantly ($p=0.036$).

Conclusions: Data from our work suggest that iron deficiency could affect many metabolic pathways, including vitamin B12, folate, and lipids. These changes normalise after iron therapy, even in women with baseline low levels of serum vitamin B12. Healthcare practitioners should be aware of these changes in IDA management. The mechanisms controlling these changes remain to be explained, but they are probably related to the control of iron homeostasis (iron deficiency mediated stimuli).

Keywords

Anemia, iron, vitamin B12, folate.

Introduction

The relationship between iron deficiency and vitamin B12 deficiency was recognized several decades ago [1–4]. Vitamin B12 deficiency is relatively common, but the majority of subjects in epidemiological studies have subclinical vitamin B12 deficiency, and do not present the classic signs of clinical deficiency [1, 5]. However, patients with subnormal vitamin B12 levels and normal vitamin B12 related metabolites (homocysteine and methylmalonic acid) are not uncommon and are often found in pregnancy, iron deficiency, HIV infection, etc. [1, 2, 6, 7]. Such patients must be differentiated from subjects with iron deficiency and low serum cobalamin levels, but a real cobalamin deficiency [3, 7–9]. Moreover, low-normal or borderline values of cobalamin (150-200 pmol/l or 250 pmol/l) are far more common (for instance, 15-20% of western population), especially in the elderly [1, 2, 5, 7, 10, 11].

Among the elderly, combined iron deficiency and vitamin B12 deficiency have been found in some of these subjects using vitamin metabolic markers [7, 12]. By contrast, iron deficiency and low levels of serum vitamin B12 with normal metabolic markers were often found mostly in young adults [1, 7, 13].

Combined deficiency is important in clinical practice owing to its relationship with malabsorption related syndromes, such as pernicious anemia, Helicobacter pylori infection, celiac disease, and gastrectomy/gastroplasty [14]. These disorders induce refractory or unexplained iron deficiency, a frequent cause for consultation in Hematology. The etiologic approach to these patients with combined deficiency is different, and the aforementioned causes should be rapidly confirmed or discarded [15].

Since iron deficiency anaemia is the most frequent cause of anaemia [16], and since clinical and diagnostic outcomes are conditioned by coexisting vitamin B12 deficiency, a flowchart to decide whether to perform vitamin assay for normocytic or microcytic anaemic patients was necessary. Using age over 60 years as a cut-off, most patients with combined deficiency could be identified [7] . But, as stated above, low vitamin B12 with normal vitamin related metabolites was often found in iron deficiency anaemia below this age[1, 7, 13].

The aim of this work was to investigate vitamin B12/folate changes in young adults and during treatment of iron deficiency anemia (IDA) with pharmacological iron. In addition, changes in other biochemical variables were also evaluated.

Materials and methods

Subjects

Patients were recruited at Complejo Hospitalario de Toledo, Spain. Inclusion criteria were: Caucasian women aged 18-40 years, haemoglobin ≤ 110 g/L, mean corpuscular volume (MCV) < 98 fL, erythrocyte sedimentation rate (ESR)< 40 mm Hg, transferrin saturation <15% and ferritin < 20 µg/l, or if ferritin was 20-50 µg/l, also soluble transferrin receptor (sTfR) > 5 mg/l. Exclusion criteria were: amenorrhoea, menopause, smoker, pregnancy, breast-feeding, non-Caucasian, thalassemia, iron-metabolism related diseases such as haemochromatosis, autoimmune diseases, chronic gastrointestinal diseases (inflammatory bowel disease, gastric ulcers, coeliac disease, Crohn's disease), neoplastic diseases, renal disease or hormone related diseases independent of iron-deficiency anaemia, C-reactive protein > 5 mg/L, creatinine levels > 1,0 mg/L (120 µmol/L), abnormal liver tests (> 2 times normal), blood donation in the past 3 months or currently taking pharmacological iron supplements.

Over 18 months, 84 women who met the inclusion criteria were interviewed by telephone to assess all exclusion criteria. After the exclusion criteria were applied, 36 women agreed to participate; one subject abandoned the study due to a change of residence.

The Complejo Hospitalario de Toledo Clinical Research Ethics Committee, Spain and the Spanish National Research Council Ethics Committee (Bioethics subcommittee) approved the present study. The study was conducted according to guidelines laid down in the Declaration of Helsinki, and participants gave signed written consent.

Study Protocol

On their first visit, patients were prescribed an eight-week supply of pharmacological iron in the form of ferrous sulphate tablets: 1 tablet (80 mg Fe) daily if haemoglobin > 100 g/L or 2 tablets a day (160 mg Fe) if haemoglobin <100

g/L. Patients were instructed to take the tablets in fasting conditions with water or orange juice.

A follow-up visit was scheduled 9 weeks after the baseline visit, one week after completing the prescribed pharmacological treatment to allow iron levels to stabilise.

If patients had not recovered from anaemia and / or iron deficiency after the first 8-week cycle of treatment (haemoglobin> 120 g/L and ferritin ≥ 15 μg/L), a further 8 weeks of treatment was prescribed, with a second follow-up visit scheduled one week after finishing the second cycle of treatment.

The compliance of the study was assessed by questionnaires and a personal interview in each visit. Patients were asked about the number of tablets that were taken throughout treatment.

Complete response was defined by normalisation of Hb (Hb > 120 g/l) and serum ferritin ≥ 15 μg/l. Incomplete response was defined by normalization of Hb (Hb > 120 g/l) but serum ferrritin < 15 μg/l, or an increase of Hb ≥ 20 g/l.

Blood samples and anthropometric measurements were taken and clinical, gynaecological, and dietary questionnaires were completed at every visit. Body weight was measured at baseline using a Seca scale (to a precision of 100 g) and height was measured (at baseline only) with a stadiometer incorporated into the scale. Body mass index (BMI) was calculated as weight/height squared (kg/m^2). Blood samples were collected between 8:00 and 10:00 by venepuncture after a 12-hour fasting period. Serum was obtained after centrifugation (for 5 minutes at 1000g).

Dietary assessment

Baseline food, energy and nutrient intakes were assessed with a 72-hour detailed dietary report previously validated in iron deficient women (17,18). The possible food intake changes throughout the study were monitored using a food frequency questionnaire.

Haematological and Biochemical assays

Total blood cell counts and ESR were determined in whole blood following standard laboratory techniques using the Beckman Coulter LH780

Analyzer (Beckman Coulter, Brea, California). Serum iron, serum ferritin, total iron binding capacity, serum vitamin B12, serum folate, sTfR, and other biochemical variables were determined by modular analysers (Elecsys and Modular DP, Roche Diagnostics, Mannheim, Germany).

Statistical Analysis

Data analysis was carried out using R software (version 3.0.1). Non-normally distributed continuous variables were log-transformed and results are presented as means with their standard deviations. Differences between baseline and end of treatment for iron biomarkers and biochemical parameters were compared using paired t-tests. Differences between groups for hematological parameters according to folate and vitamin B12 levels were performed by analysis of variance (ANOVA). Pearson correlation test was performed to study the correlation between the changes in Hb and biochemical parameters. Values of $p < 0.05$ were considered significant. Ordinal variables were compared using Wilcoxon Signed Rank Test.

Results

Thirty-five women were included and completed the study. The subjects were aged 35.3± 5.3 years. BMI was 26.1 ± 4.6 kg/m^2 at baseline and did not significantly vary throughout treatment (26.0 ± 4.5 kg/m^2 at end of treatment). No significant changes were seen in food intake between baseline and end of treatment, measured by the food frequency questionnaire, except for "Fruit juices at breakfast" which showed an increased consumption (median increased from 0 to 1) (p=0.015) and "Other dairy products at breakfast" which decreased (although median is 0 at both timepoints) (p=0.022) (table1).

Table 2 shows the changes in blood cell counts and iron biomarkers from baseline to the end of treatment. Incomplete or complete response was observed in 97.2 % (at 4-month follow-up, 77% complete responses and 20% incomplete responses).

Treatment with iron increased significantly serum folate and vitamin B12 from baseline (table 2). In vitamin B12 this increase was observed in patients with vitamin B12 levels ≤200 pmol/l (6 cases, 17.1%, including 5 with serum folate ≤13

nmol/l). At the end of the study serum vitamin B12 was higher than 200 pmol/l in all patients (table 3).

Table 4 shows changes from baseline to the end of treatment for other biochemical parameters. Significant increases were seen for glucose ($p=0.012$), uric acid ($p<0.001$), total cholesterol ($p=0.023$), HDL-cholesterol ($p=0.026$), and bilirubin ($p<0.001$). Urea decreased significantly ($p=0.036$), and creatinine, proteins, albumin, triglycerides and LDL-cholesterol had no significant changes.

Patients with B12 <200 pmol/L (n=6) did not show significant differences in hematologic parameters compared to those patients with only iron deficiency. However, patients with folate <13 nmol/L (n=8) presented lower hemoglobin, MCV, MCHC, serum iron and transferrin saturation, and higher sTrR levels compared to the other patients (table 5). No correlation between the Hb increase and the increases in the biochemical parameters were observed. However, serum vitamin B12 and serum folate changes were correlated ($r =0. 406$; $p= 0.017$).

Discussion

The purpose of this study was to investigate serum vitamin B12 and folate changes during treatment of IDA with ferrous sulphate. It was found that the recovery from IDA was accompanied by an increase in levels of vitamin B12, especially in cases with the lowest levels of serum vitamin B12, and folic acid. Moreover other biochemical significant variations were observed. For instance, glucose, total cholesterol and HDL cholesterol increased. These findings should be taken into account by healthcare practitioners dealing with IDA management.

In this group, energy intake was adequate for women of their age and activity levels. Protein intake was marginally higher than recommended, whilst carbohydrate intake was lower and lipid intake was higher than recommended. Vitamin intake was higher than the recommended intake for vitamins B1, B2, B6, B12, C, and niacin, whilst for folic acid and vitamins D and E it did not reach the recommended value [19].

In a previous study, low vitamin B12 levels (< 200 pmol/l) were found in approximately 20% of patients with IDA, including patients with vitamin B12 deficiency, demonstrated by an increase in homocysteine. Characteristically, the

patients included in that group were over 60 years of age. The remaining group of patients showed subnormal levels of serum vitamin without metabolic changes of vitamin B12 deficiency. This second group included the youngest patients who were characterised by IDA with low vitamin B12, low holotranscobalamin, and normal homocysteine and methylmalonic acid, excluding vitamin B12 deficiency [13].

In the present study a group of young adult women with IDA, in whom other pathologies were excluded, were investigated before and after oral iron therapy. The results demonstrated that serum vitamin B12 increased with iron therapy. The increase was significant in patients with the lowest levels of serum vitamin (<200 pmol/l). Serum folate also significantly increased, but the baseline levels and the values at the end of the study were within the reference range for all patients. Other biochemical variables also significantly changed, but most of the values were also within the reference range. These metabolic changes did not show a relationship with the Hb increase; however serum vitamin B12 and folate were positively correlated.

Changes in B12 levels upon treatment of IDA have been studied previously with mixed conclusions. Whilst Roberts et al. and Akhmeteli et al. [20,21] found B12 levels to be unchanged in IDA patients during iron therapy, in agreement with our results, Harrison [22] found that B12 levels increased with pharmacological iron treatment. Several studies, however, concur in finding that B12 levels are reduced in IDA patients [7,23,24], with a similar percentage of patients with low B12 levels (17.8%) as in our previous studies [7].

With regards to folic acid, several studies are in agreement with the present study; one report [25] indicates that low folate levels related to iron deficiency remitted in patients with iron therapy whilst on a low folic acid diet and other observed that iron treatment alone corrected abnormalities in the distribution of folate between plasma and erythrocytes [26]. Furthermore, a high prevalence of serum folate deficiency was reported in a group of 50 IDA patients that improved with iron treatment [20]. In animal models contradictory results have been observed. In this line, studies in rats found secondary folate deficiency was induced by dietary iron deficiency [27–29], however another study did not find such a relationship [30].

Folate and iron are absorbed in the same areas of intestine (upper small intestine). Interaction between iron and folate absorption may be provided by the haem carrier protein 1, which is both a mammalian haem transporter and a proton-coupled folate transporter; although its affinity for haem was lower than to folate, it has been suggested that its affinity could be increased in IDA [31]. By contrast, vitamin B12 absorption is limited to terminal ileum where direct competition with iron absorption is minimal.

In this regard, our study also observed changes in other biochemical variables, especially a decrease in lipid variables. As with the present study, other assays have found lipid metabolism to be affected by iron deficiency. Choi et al. [32] observed, in teenage girls, that serum total cholesterol concentration and serum triglycerides were significantly lower in severely anaemic subjects than in controls, and that both total cholesterol and triglyceride levels were elevated after iron supplementation, whereas in our assay, although cholesterol levels increased after treatment, triglycerides did not change. Ozdemir et al. [33] studied premenopausal women with IDA, finding that total and LDL cholesterol in the anaemic women were lower than in non-anaemic controls, and that levels increased after treatment, but remained lower than in control subjects. In contrast, our study found HDL levels increased but LDL levels did not change. These authors also hypothesize that low iron status in premenopausal may have a protective effect against atherosclerotic heart disease by affecting lipid metabolism.

Mixed results are seen from studies in experimental models. In rats, Stangl and Kirchgessner [34] found that iron depleted rats had lower serum lipoproteins. Bristow-Craig et al. [35] found cholesterol and triglycerides to be lower in rats fed a low iron diet, and Cunnane and McAdoo [36] found fatty acid metabolism to be mildly impaired in moderately iron-deficient rats. However other studies have associated iron deficiency with increased levels of serum triglycerides, and studies in iron-deficient rats on triglyceride synthesis in the liver do not provide a clear picture [37-42].

Possible interrelationship between vitamin B12, folic acid and iron in erythropoiesis has been proposed [24]. Recent discoveries have improved our knowledge of iron homeostasis. Hepcidin, a hepatic protein, is the key regulator of iron metabolism. In uncomplicated IDA, hepcidin production is decreased. As a

consequence, intestinal iron absorption and macrophage and hepatocyte iron release are raised. On the contrary, an increase in iron stores stimulates the production of hepcidin and, therefore, absorption and release of iron decrease. This fine mechanism of iron homeostasis maintains an adequate iron balance.

Data from our work suggest that iron deficiency could affect many metabolic pathways, including vitamin B12, folate, and lipid. These changes normalise after iron therapy. In this regard, vitamin B12, folate and lipid metabolisms may have been influenced by iron deficiency stimuli, mediated by hepcidin itself or by other substances [43-46].

In conclusion, serum vitamin B12 and folate levels decreased in iron deficiency and increased during treatment with pharmacological iron. The increase in levels of vitamin B12 was significant in cases with the lowest levels of serum vitamin B12. Other biochemical significant variations were observed, especially in lipid metabolism (total cholesterol and HDL cholesterol raised). Healthcare practitioners should be aware of these changes in IDA management. The mechanisms controlling these changes remain to be explained, but they are probably related to the control of iron homeostasis.

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Conflict of interest:

The authors declare that they have no conflict of interest.

References

1. Carmel R. Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: a critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II. Am J Clin Nutr 2011; 94:348S–358S.

2. Carmel R. How I treat cobalamin (vitamin B12) deficiency. Blood 2008; 112:2214–21.

3. Chanarin I The Megaloblastic Anaemias, 3rd ed. Blackwell Sci Pub, Oxford 1990.

4. Craig GM, Elliot C, Hughes KR Masked vitamin B12 and folate deficiency in the elderly. Br J Nutr 1985; 54:613–9.

5. Metz J A high prevalence of biochemical evidence of vitamin B12 or folate deficiency does not translate into a comparable prevalence of anemia. Food Nutr Bull 2008; 29:S74–85.

6. Remacha AF, Cadafalch J, Sardà P, et al. Vitamin B-12 metabolism in HIV-infected patients in the age of highly active antiretroviral therapy: role of homocysteine in assessing vitamin B-12 status. Am J Clin Nutr 2003; 77:420–4.

7. Remacha AF, Sardà MP, Canals C, et al. Combined cobalamin and iron deficiency anemia: a diagnostic approach using a model based on age and homocysteine assessment. Ann Hematol 2013; 92:527–31.

8. Oosterhuis WP, Niessen RW, Bossuyt PM, et al. Diagnostic value of the mean corpuscular volume in the detection of vitamin B12 deficiency. Scand J Clin Lab Invest 2000; 60:9–18.

9. Menzie CM, Yanoff LB, Denkinger BI, et al. Obesity-related hypoferremia is not explained by differences in reported intake of heme and nonheme iron or intake of dietary factors that can affect iron absorption. J Am Diet Assoc 2008; 108:145–8.

10. Harper JW, Holleran SF, Ramakrishnan R, et al. Anemia in celiac disease is multifactorial in etiology. Am J Hematol 2007; 82:996–1000.

11. Varela-Moreiras G, Murphy MM, Scott JM Cobalamin, folic acid, and homocysteine. Nutr Rev 2009; 67 Suppl 1:S69–72.

12. Green R Indicators for assessing folate and vitamin B12 status and for monitoring the efficacy of intervention strategies. Food Nutr Bull 2008; 29:S52–63; discussion S64–6.

13. Remacha AF, Sardà MP, Canals C, Queraltò JM, Zapico E, Remacha J, Carrascosa C. Role of serum holotranscobalamin (holoTC) in the diagnosis of patients

with low serum cobalamin. Comparison with methylmalonic acid and homocysteine. Ann Hematol 2014; 201; 93:565-9.

14. Hershko C, Skikne B. Pathogenesis and management of iron deficiency anemia: emerging role of celiac disease, helicobacter pylori, and autoimmune gastritis. Semin Hematol 2009; 46:339–50.

15. Hershko C, Camaschella C How I treat unexplained refractory iron deficiency anemia. Blood 2014; 123:326–33.

16. Kassebaum NJ, Jasrasaria R, Naghavi M, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood 2014; 123:615–24.

17. Blanco-Rojo R, Pérez-Granados AM, Toxqui L, González-Vizcayno C, Delgado MA,Vaquero MP. Efficacy of a microencapsulated iron pyrophosphate-fortified fruit juice: a randomised, double-blind, placebo-controlled study in Spanish iron-deficient women. Br J Nutr 2011; 105: 1652-9.

18. Toxqui L, Pérez-Granados AM, Blanco-Rojo R, Wright I, González-Vizcayno C, Vaquero MP. Effects of an iron or iron and vitamin D-fortified flavored skim milk on iron metabolism: a randomized controlled double-blind trial in iron-deficient women. J Am Coll Nutr 2013;32:312-20.

19. Cuervo M, Corbalán M, Baladía E, Cabrerizo L, Formiguera X, Iglesias C, Lorenzo H, Polanco I, Quiles J, Romero de Avila MD, Russolillo G, Villarino A, Alfredo Martínez J. [Comparison of dietary reference intakes (DRI) between different countries of the European Union, The United States and the World Health Organization] Nutr Hosp. 2009;24:384-414.

20. Roberts PD, St John DJ, Sinha R, et al. Apparent folate deficiency in iron-deficiency anaemia. Br J Haematol 1971; 20:165–76.

21. Akhmeteli KT, Eradze TS, Tushurashvili PR, Margvelani GP [Vitamins C, B12 and folic acid in latent iron deficiency]. Georgian Med News 2005; 11:109–11.

22. Harrison RJ Vitamin B 12 levels in erythrocytes in hypochromic anaemia. J Clin Pathol 1971; 24:698–700.

23. Areekul S, Ratanabanangkoon K, Hathirat P, et al. Vitamin B12 and vitamin B12 binding proteins in iron deficiency anaemia. Southeast Asian J Trop Med Public Health 1978; 9:510–5.

24. Koury MJ, Ponka P New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr 2004, 24:105–31.

25. Velez H, Restrepo A, Vitale JJ, Hellerstein EE Folic acid deficiency secondary to iron deficiency in man. Remission with iron therapy and a diet low in folic acid. Am J Clin Nutr 1966; 19:27–36.

26. Omer A, Finlayson ND, Shearman DJ, et al. Plasma and erythrocyte folate in iron deficiency and folate deficiency. Blood 1970; 35:821–8.

27. Vitale JJ, Restrepo A, Velez H, et al. Secondary folate deficiency induced in the rat by dietary iron deficiency. J Nutr 1966; 88:315–22.

28. Toskes PP, Smith GW, Bensinger TA, et al. Folic acid abnormalities in iron deficiency: the mechanism of decreased serum folate levels in rats. Am J Clin Nutr 1974; 27:355–61.

29. Kochanowski B a, Smith a M, Picciano MF, Sherman AR (1983) Folate depletion secondary to iron deficiency in the neonatal rat. J Nutr 113:2471–8.

30. Burns DG, Spray GH Normal folic acid metabolism in iron-deficient rats. Br J Nutr 1969; 23:665–70.

31. Laftah AH, Latunde-Dada GO, Fakih S, et al. Haem and folate transport by proton-coupled folate transporter/haem carrier protein 1 (SLC46A1). Br J Nutr 2009; 101:1150–6.

32. Choi JW, Kim SK, Pai SH. Changes in serum lipid concentrations during iron depletion and after iron supplementation. Ann Clin Lab Sci 2001; 31:151–6.

33. Ozdemir A, Sevinç C, Selamet U, Türkmen F (2007) The relationship between iron deficiency anemia and lipid metabolism in premenopausal women. Am J Med Sci 2007; 334:331–3.

34. Stangl GI, Kirchgessner M Different degrees of moderate iron deficiency modulate lipid metabolism of rats. Lipids 1998; 33:889–95.

35. Bristow-Craig HE, Strain JJ, Welch RW Iron status, blood lipids and endogenous antioxidants in response to dietary iron levels in male and female rats. Int J Vitam Nutr Res 1994; 64:324–329.

36. Cunnane SC, McAdoo KR. Iron intake influences essential fatty acid and lipid composition of rat plasma and erythrocytes. J Nutr 1987; 117:1514–1519.

37. Amine EK, Hegsted DM. Iron deficiency lipemia in the rat and chick. J Nutr 1971; 101:1575–82.

38. Amine E, Desilets E, Hegsted D. Effect of dietary fats on lipogenesis in iron deficiency anemic chicks and rats. J Nutr 1976; 106:405–411.

39. Sherman AR Serum lipids in suckling and post-weanling iron-deficient rats. Lipids 1979; 14:888–92.

40. Sherman AR, Guthrie HA, Wolinsky I, Zulak IM Iron deficiency hyperlipidemia in 18-day-old rat pups: effects of milk lipids, lipoprotein lipase, and triglyceride synthesis. J Nutr 1978; 108:152–62.

41. Ahmed U, Latham PS, Oates PS Interactions between hepatic iron and lipid metabolism with possible relevance to steatohepatitis. World J Gastroenterol 2012; 18:4651–8.

42. Davis MR, Rendina E, Peterson SK, et al. Enhanced expression of lipogenic genes may contribute to hyperglycemia and alterations in plasma lipids in response to dietary iron deficiency. Genes Nutr 2012; 7:415–25.

43. Meynard D, Babita JL, Lin HY. The liver: conductor of systemic iron balance. Blood 2014; 123: 168-176.).

44. Ahmed U, Oates PS Dietary fat level affects tissue iron levels but not the iron regulatory gene HAMP in rats. Nutr Res 2013; 33:126–35.

45. Kamei A, Watanabe Y, Ishijima T, et al. Dietary iron-deficient anemia induces a variety of metabolic changes and even apoptosis in rat liver: a DNA microarray study. Physiol Genomics 2010; 42:149–56.

46. Kamei A, Watanabe Y, Kondo K, et al. Influence of a short-term iron-deficient diet on hepatic gene expression profiles in rats. PLoS One 2013; 8:e65732.

Table 1. Daily energy and nutrient intake of the study participants at baseline

	Value	Recommended intake
Energy (kcal)	2194 ± 415	
Proteins (% energy)	16.9 ± 2.9	
Carbohydrates (% energy)	38.9 ± 6.4	
Lipids (% energy)	40.7 ± 6.4	
Cholesterol (mg/day)	338± 158	<300
Iron (mg/day)	16.5 ± 5.7	18
Vitamin B1 (mg/day)	1.7 ± 0.6	0,9
Vitamin B2 (mg/day)	2.0 ± 0.8	1,4
Vitamin B6 (mg/day)	2.6 ± 0.9	1,6
Vitamin B12 (μg/day)	10.0 ± 17.1	2,0
Folic Acid (μg/day)	296 ± 118	400
Vitamin C (mg/day)	144± 76	60
Vitamin D (μg/day)	2.8 ± 2.5	5
Vitamin E (mg/day)	9.0 ± 3.9	12

Values are presented as means with their standard deviations. Recommended daily intake for Spanish women, age 20-39 y [19].

Table 2. Changes in blood cell counts and iron biomarkers after iron treatment

	Baseline	End of treatment	Paired t-test, p value
Hemoglobin (g/L)	99.6 ± 9.7	132.3 ± 10.8	<0.001
MCV (fL)	73.0 ± 6.8	86.6 ± 5.7	<0.001
MCHC (g/L)	319.9 ± 9.5	337.2 ± 7.2	<0.001
RDW	17.6 ± 1.8	17.9 ± 4.1	NS
Serum ferritin (µg/L)	4.25± 2.0	23.8 ± 18.6	<0.001
Serum iron (mcg/dL)	24.7 ± 8.0	70.1 ± 31.0	<0.001
TIBC (mcg/dL)	479 ± 52	390 ± 55	<0.001
Transferrin saturation (%)	5.2 ± 1.7	18.2 ± 7.8	<0.001
sTfR (g/L)	8.62 ± 3.56	3.68 ± 1.39	<0.001
Platelets (x10^9/L)	297 ± 80	246 ± 54	<0.001

Values are presented as means with their standard deviations. Comparisons between baseline and end of treatment were measured using paired t-test.

Table 3. Changes in serum folate and serum vitamin B12 after iron treatment

	N (%)	Baseline	End of study	Increase %	Paired t-test, p value
Serum vitamin B12					
≤200 pmol/l	6 (17.1)	166 ± 24	248 ± 31	49.90	p<0.01
>200 pmol/l	29 (82.9)	341 ± 96	372 ± 124	9.20	NS
Total	35 (100)	311 ± 110	351 ± 123	12.90	p<0.05
Serum folate					
≤13 nmol/l	8 (22.9)	10.6 ± 1.6	16.7 ± 5.6	58.20	p<0.05
>13 nmol/l	27 (77.1)	19.6 ± 6.3	21.8 ± 7.8	10.90	p<0.05
Total	35 (100)	17.5 ± 6.8	20.6 ± 7.6	17.50	p<0.01

Values are presented as means with their standard deviations. Comparisons between baseline and end of treatment were measured using paired t-test.

Table 4. Changes in biochemical parameters after iron treatment

Biochemical parameters	Baseline	End of treatment	Paired t-test, p value
Glucose (mg/dL)	83.09 ± 9.34	88.56 ± 9.17	0.012
Urea (mg/dL)	30.12 ± 6.25	28.09 ± 4.46	0.036
Creatinine (mg/dL)	0.73 ± 0.09	0.74 ± 0.10	NS
Uric acid (mg/dL)	3.39 ± 0.66	3.94 ± 0.87	<0.001
Proteins (g/dL)	7.10 ± 0.45	7.17 ± 0.40	NS
Albumin (g/dL)	4.50 ± 0.26	4.58 ± 0.27	NS
Cholesterol (mg/dL)	172.03 ± 28.46	179.65 ± 27.61	0.023
Triglycerides (mg/dL)	69.21 ± 33.74	64.76 ± 30.74	NS
HDL-chol (mg/dL)	60.31 ± 12.61	61.03 ± 12.76	0.026
LDL-chol (mg/dL)	101.95 ± 21.25	106.22 ± 24.82	NS
Bilirubin (mg/dL)	0.39 ± 0.13	0.56 ± 0.26	<0.001

Values are presented as means with their standard deviations. Comparisons between baseline and end of treatment were measured using paired t-test.

Table 5. Hematological parameters according to folate levels at baseline

	Folate ≤13 nmol/L	Folate >13 nmol/L	ANOVA, p value
Vitamin B12 pmol/l	206 ± 70	341 ± 103	0.002
Hb (g/L)	93 ± 14	102 ± 7	0.019
MCV (fL)	68 ± 5	74 ± 7	0.031
MCHC (g/L)	314 ± 17	322 ± 7	0.034
RDW	18 ± 2	17 ± 2	NS
Serum ferritin (µg/L)	4 ± 1	5 ± 2	NS
Serum iron (mcg/dl)	19 ± 7	26,75 ± 7,41	0.011
TIBC (mcg/dl)	469 ± 48	480 ± 54	NS
Transferrin saturation (%)	4 ± 2	6 ± 2	0.031
sTfR (g/L)	11 ± 5	8 ± 3	0.024
Platelets (x109/L)	324 ± 117	285 ± 64	NS

Values are presented as means with their standard deviations. Comparisons between groups by ANOVA.